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The Stress Response to an Acute Heavy Resistance Exercise Protocol

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B.S. Bentley University, 2007

A Thesis
Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science
at the
University of Connecticut 2014

Approval Page

Master of Science Thesis

The Stress Response to an Acute Heavy Resistance Exercise Protocol

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2014

ACKNOWLEDGEMENTS

Dr. Kraemer

Thank you for your guidance and support and most importantly for taking a chance on me. To work under you, as part of the Human Performance Laboratory, was truly a special opportunity.

Dr. Lee

Thank you for your help in developing the concept for this manuscript, for teaching me the biochemical techniques needed to complete and for all of the support and insight you provided along the way.

Dr. Maresh & Dr. Volek

Thank you for your positive input, helpful criticism, and the many opportunities made possible by your hard work and leadership.

Shawn Flanagan, Jenna Apicella, Colleen Munoz & Daniel Friedenreich

Simply put, you were all invaluable in completing this project, and I could not have done it without you. I cannot thank you enough for taking the time to help me, despite being extremely busy yourselves.

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ABSTRACT

Exercise has been shown to induce significant stress and a subsequent inflammatory response characterized by changes in circulating leukocyte populations and inflammatory cytokines and has been well documented following aerobic exercise. Research in regards to the effects of resistance exercise on the stress induced inflammatory response is less prevalent and has shown divergent results, likely due to differences in the exercise protocols used, the time points measured and the subjects involved. Therefore, the purpose of this investigation was to analyze the stress response to an acute heavy resistance exercise protocol (AHREP) in resistance-trained men. Specifically, we examined effects on circulating leukocytes, inflammatory cytokines, and extracellular heat shock protein 70 (HSP70). Ten resistance-trained men completed an AHREP consisting of high intensity back squat for six sets of 10 repetitions. Blood draws were taken before and immediately after the protocol, and at 15, 30, 60 and 120 minutes into recovery. Additional blood draws were taken 24, 48 and 72 hours into recovery. Increases in lactate, cortisol and creatine kinase following the AHREP confirmed the stressful and damaging nature of the protocol. Significant changes were observed in circulating leukocytes and extracellular HSP 70, though no significant changes were observed in the pro-inflammatory cytokines TNF- α , IL-1, IL-8 and IL-12p70, nor in the anti-inflammatory cytokines IL-6 and IL-10. Our findings help further elucidate the inflammatory response to resistance exercise stress in resistance-trained men. Furthermore, our findings show that

resistance exercise can induce an increase in extracellular HSP70 provided the protocol is sufficiently stressful.

Chapter 1: Introduction

Exercise exerts a multifaceted stress upon the body eliciting an inflammatory response in an attempt to repair damage and restore homeostasis. The inflammatory response is characterized by an increase in circulating leukocytes and the extravasation of leukocytes into tissue. The function of these leukocytes is influenced by cytokines, small cellular signaling molecules, which increase as part of the inflammatory process. In contrast to the inflammatory response to infection, in which the pro-inflammatory cytokines TNF- α and IL-1 predominate, the response to exercise is largely mediated by the actions of IL-6(46). During exercise, IL-6 is released from contracting muscles inducing the release of the anti-inflammatory cytokines IL-10 and IL-1ra(38). The downregulation of the adaptive immune response and the inhibition of IL-1 β synthesis and secretion are essential components of the transition from the early pro-inflammatory period to the subsequent anti-inflammatory period(28, 33, 46).

In addition to its intracellular role as cell chaperone, heat shock protein 70 can be secreted into the extracellular environment where it exhibits cytokine-like behaviors, leading some researchers to refer to it as a “chaperokine(1, 34, 50).” Extracellular heat shock protein 70 (eHSP70) has been shown to increase in the blood following aerobic exercise(8, 27, 49), though the stimulus for its release, and its role in the inflammatory process, have yet to be elucidated in vivo. In vitro studies have shown that norepinephrine can induce eHSP70 secretion by neutrophils and that eHSP70 can stimulate inflammatory cytokine production in peripheral blood mononuclear cells(15, 21). Thus, eHSP70 may serve as a

mediator of immune and neuroendocrine integration in the inflammatory response.

The effect of resistance exercise on plasma concentrations of the key inflammatory cytokines is inconclusive, with responses varying between investigations(7, 20, 22-24, 39, 43, 47, 48). Additionally, investigations employing resistance exercise have failed to elicit the eHSP70 response observed in response to aerobic exercise(20, 37). The resistance exercise protocols employed vary between investigations and may not have been sufficiently stressful to elicit the inflammatory response. Therefore, the purpose of this investigation is to examine the leukocyte, cytokine and eHSP70 components of the inflammatory response to an acute high intensity resistance exercise protocol (AHREP).

The acute high intensity resistance exercise protocol (AHREP) was designed by our group to cause exhaustion and systemic stress through high intensity and volume coupled with short rest periods on the dynamic squat, which activates large amounts of muscle mass throughout the body. The ability of the AHREP to induce metabolic, adrenal, and mechanical stress has been reliably demonstrated by elevations in lactate, cortisol, epinephrine, norepinephrine, myoglobin and creatine kinase(3, 4, 11, 13, 25, 31, 32, 41, 51). Furthermore, similar protocols have been shown to induce significant stress in resistance trained subjects, like those recruited for this investigation. Thus, the AHREP is an ideal stimulus for investigation of the inflammatory response to resistance exercise-induced stress.

Research Questions and Hypotheses

1. How do circulating leukocyte counts change in response to resistance exercise?

Increases in circulating leukocytes in response to aerobic exercise are well documented. Resistance exercise, though less studied, has generally been shown to induce similar increases. We expect to see increases in all leukocytes immediately after the protocol, neutrophilia at all acute time points, and lymphopenia following the initial increase. The influence of resistance exercise on monocyte counts is less clear, however, we expect monocytosis immediately following the protocol followed by either sustained monocytosis or a return to baseline values. Eosinophils will likely be elevated immediately after the AHREP and then return to baseline values.

2. Does resistance exercise influence the concentrations of IL-1 β , IL-6, IL-8, IL-10, IL-12p70 and TNF- α ?

Resistance exercise generally does not impact IL-1 β , IL-8, IL-12p70 or TNF- α plasma concentrations, though exceptions have been observed. IL-8 is believed to be intensity dependent, and therefore may be expected to increase following the taxing AHREP protocol. We do not expect to see changes in these four cytokines, but of the four, IL-8 is most likely to change.

Changes in IL-6 and IL-10 have been observed following resistance exercise, though a nearly equal number of investigations have shown no change. Additionally, increases in these cytokines have been demonstrated at different

points during recovery. IL-6 has only been shown to increase in untrained subjects, and to our knowledge, only one study has examined it in resistance-trained men, where changes were not observed. IL-10 increases have been demonstrated in trained and untrained men. One study demonstrated that IL-10 secretion increased after several weeks of training. Given that we are using resistance trained subjects, we expect an increase in IL-10 and may observe an increase in IL-6.

3. Does resistance exercise induce an increase in eHSP70?

Increases in eHSP70 have been observed following numerous aerobic exercise protocols, however, previous investigations employing resistance exercise have shown no significant change. The resistance exercise used in the two previous investigations consisted solely of elbow flexor eccentrics, which may not have generated sufficient systemic stress. Given the demonstrated ability of the AHREP to induce substantial stress and damage, we expect to see an increase eHSP70, though we are unsure as to the magnitude or timing of the response.

Chapter 2: Literature Review

The Stress of the AHREP

Lactate

During resistance exercise, the body relies on anaerobic metabolism to synthesize the ATP needed to sustain muscle contraction. The formation of lactate from pyruvate in fast glycolysis results in an accumulation of blood lactate. The magnitude of lactate accumulation is dependent on the acute program variables of the given resistance exercise protocol. High volume and short rest period resistance exercise using moderate to heavy loads has been shown to maximize the lactate values(30). The amount of muscle mass recruited during exercise also affects the magnitude of lactate increase(30).

Observed plasma lactate values are impacted by both the magnitude of the lactate response and the rate of lactate clearance, both of which are affected by anaerobic training. When compared to untrained individuals anaerobically trained individuals exhibit higher lactate clearance rates and a lower lactate response to an absolute workload(3). When relative loading is used, the trained and untrained individuals exhibit similar lactate responses(3).

Previous investigations using the AHREP have consistently observed significant increases in lactate, with peak lactate levels reported immediately after the protocol and gradually decreasing throughout recovery (6, 8, 17, 32, 58, 72, 76). In investigations reporting a return to baseline values, this return occurred two to three hours into recovery. Peak lactate values in these investigations ranged from 11.5 ± 0.9 to 13.4 ± 0.5 mmol/L, corresponding to 10

and 8 fold increases, respectively (6, 8, 17, 32, 58, 72, 76).

Catecholamines

As part of the stress response to resistance exercise, epinephrine and norepinephrine are released from the adrenal medulla. Additionally, norepinephrine is secreted from sympathetic neurons. Following secretion, the catecholamines affect numerous physiological processes including substrate utilization, force production, and blood flow. Catecholamines also induce acute leukocytosis through demargination (60) and have been shown to stimulate secretion of eHSP70 from immune cells(20).

Like lactate, the magnitude of the catecholamine response to resistance exercise is dependent on the intensity, volume, and rest periods of the protocol, and the amount of muscle mass stimulated(30). Elevated catecholamines can be observed prior to the onset of exercise as part of an “anticipatory response” (17), as well as during and immediately after exercise(31). Resistance training can influence this adrenergic response as trained men experience greater catecholamine increases than untrained men in response to acute resistance exercise(31).

Several investigations employing the AHREP have observed a significant catecholamine response(6, 15, 17). In these investigations peak epinephrine levels ranged from approximately 2000- 4000 pmol/L while peak norepinephrine levels from approximately 10 – 14 nmol/L. Prior to beginning the protocol, an anticipatory response was reported by one investigation, observing 94.5 and

255% increases in epinephrine and norepinephrine, respectively(17).

Cortisol

Cortisol is a glucocorticoid released from the adrenal cortex in response to psychological and physiological stress. A catabolic hormone, cortisol inhibits protein synthesis and affects protein, lipid and carbohydrate metabolism by stimulating protein degradation in muscle cells, lipolysis in adipose tissue, and gluconeogenesis(14, 30). By activating the key enzyme glycogen phosphorylase cortisol also influences glycolysis. Aside from its role in metabolism, cortisol is a strong anti-inflammatory hormone, exerting significant influence on the immune and inflammatory responses to physical stress.

Both aerobic and resistance exercise modalities can elicit increases in cortisol values. Intensity and duration affect the response to aerobic exercise, with higher intensities and longer durations eliciting greater increases in cortisol values(29). In resistance exercise, the greatest cortisol response is elicited when high intensity loading, high volume and short rest periods are utilized(14, 30). Consequently, the cortisol and lactate responses to exercise are highly correlated. Following resistance exercise, cortisol values generally peak between IP and 30 minutes and gradually decline to or baseline values within 90-120 minutes(16).

Two investigations utilizing the AHREP have reported increases in cortisol. Bush et al reported a non-significant increase in cortisol at IP and a significant increase at 15 minutes post, the last time point measured(6). A longer

recovery timeline reported by Vingren et al showed an increase cortisol at IP, which peaked 20-40 minutes post and returned to baseline values by 140-300 minutes post(72). The peak cortisol value reported by Vingren et al was 1111 ± 431 nmol/L, nearly a 90% increase from the pre value of 591 ± 274 nmol/L.

Muscle Damage

The eccentric contractions utilized in resistance exercise exert a mechanical stress on the sarcomeres, leading to disruption of cytoskeleton structures and increased permeability of the sarcoplasmic membrane. While this damage can be measured directly, the indirect markers, creatine kinase (CK) and myoglobin, are commonly used to quantify the amount of muscle damage sustained. As a result of the increased membrane permeability, these cellular proteins leak out of sarcomeres and into the blood. Though both measures provide evidence of muscle damage, they do so on different timeframes due to their different molecular weights. The 16.7 kDa myoglobin molecule escapes the damaged sarcomere earlier than the larger CK molecule with a molecular weight of 43.0 kDa, resulting in an earlier appearance in the blood.

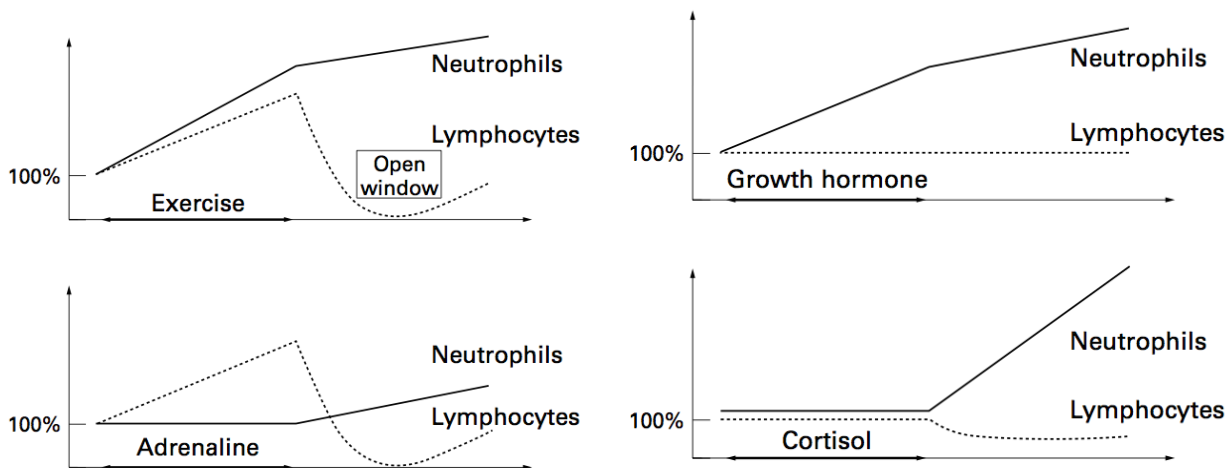
Both myoglobin and CK have been used to quantify muscle damage following the AHREP, and the results are in line with existing muscle damage literature. In Kupchak et al, resistance trained men and women exhibited elevated myoglobin immediately after completion of the protocol with values increasing throughout recovery and peaking at +120m, the last time point measured(32). Both myoglobin and CK were measured in Yamamoto et al, with myoglobin values elevated at 1 and 2 hours after the 6x10 and CK elevated at

24H into recovery(76). The CK values quickly returned by baseline by 48 hours post, likely due to the training status of the subjects.

The Effects of Stress on Circulating Leukocytes

In response to physical stress, neuro-endocrine stimulation results in a significant increase in circulating leukocyte populations. This leukocytosis results from the catecholamine induced release of leukocytes from the marginated pool and the shear stress exerted by increased cardiac output(16). Of the five leukocytes, only neutrophils, lymphocytes and monocytes are involved in the inflammatory response to physical stress. The figure below depicts the response of neutrophils and lymphocytes to exercise and hormones increased as a result of exercise.

Figure 1: The effects of exercise and hormones on circulating Neutrophils and Lymphocytes



Basophils and eosinophils are primarily responsible for the immune response to parasites and allergenic stimuli and consequently will not be included in this review.

Neutrophils

Neutrophils are an integral part of the innate immune system, and the most prevalent leukocyte in circulation, accounting for 50-70% of total leukocytes(35). The primary function of neutrophils is the phagocytosis of cellular debris through degranulation, the release of anti-microbial enzymes and metalloproteinases, and respiratory burst, the formation of reactive oxygen species(49). Chemotaxins, like IL-8, attract the neutrophils to sites of inflammation(37).

Exercise results in acute neutrophilia, though the magnitude and timing of the neutrophil response depends on the specific exercise protocol. Numerous investigations utilizing aerobic exercise have demonstrated a biphasic neutrophil response to exercise(18, 22, 39). Immediately after conclusion of the protocol, there is a large increase in circulating neutrophils due to increased catecholamines in the blood(39, 49). This increase has also correlated with increased lactate concentrations(39). The subsequent increase in neutrophils several hours later has been correlated with the increase in cortisol following the exercise protocol (22, 65). Factors affecting the magnitude of neutrophilia include exercise duration(22, 56), intensity(18) and previous exercise(57). Additionally, muscle damage incurred as a result of exercise may affect this response(53).

Studies of the neutrophil response to resistance exercise are somewhat limited when compared to that of aerobic exercise, nonetheless, a similar neutrophilic response has been demonstrated(38, 44, 54). In these studies, an increase of approximately 50% is observed immediately after the acute RE bout, and those studies with +120m timepoints show a peak change of approximately 120%. The smallest change in neutrophils from pre to IP was observed in the Mayhew et al study, which failed to show any significant increase in cortisol.

Though not identified as such, the investigation by Ramel et al seems to demonstrate the biphasic neutrophil response identified in aerobic training investigations. In this investigation, resistance trained subjects showed an increase at IP that gradually decreased at +30m, began to increase again at +60m and peaked at +120m(54). The additional studies lack enough time points to identify a biphasic response.

The AHREP has been used in several studies to examine the effects of RE on immunological variables, however, only one such study has measured changes in circulating neutrophils. In 29, non-resistance trained women, Miles et al showed a significant increase in neutrophils immediately after an AHREP(41). Of particular interest, the acute neutrophilia was due solely to an increase in segmented neutrophils, with banded neutrophils demonstrating a non-significant decrease. Such data indicate that the acute neutrophil increase is due to demargination of neutrophils and not movement from the bone marrow.

Monocytes

Like neutrophils, monocytes are a component of the innate immune response with functions including phagocytosis, cytokine production and tissue remodeling(73, 74). Monocytes also support the adaptive immune response through the presentation of antigens(35). Following recruitment to inflammatory sites via chemotaxis, monocytes differentiate into macrophages or dendritic cells.

The response of monocytes to exercise is less conclusive than that of neutrophils and lymphocytes. During and immediately following aerobic exercise, increases and no change in circulating monocytes have been found(19, 35, 63, 69). However, two to three fold increases in monocytes are commonly observed 1.5-2 hours into recovery(51). Duration and exercise intensity affect the monocyte response to aerobic exercise with shorter duration and higher intensities eliciting greater responses(19).

In contrast to aerobic exercise, resistance exercise consistently elicits an immediate monocytosis (5, 38, 41, 44, 54, 61) though the magnitude of this elevation varies greatly. Two investigations exhibited a quick return to baseline values, while three studies exhibited sustained monocytosis through their final timepoint (1.5 and 2 hours). The magnitude of increase from pre to immediately post exercise ranged from 15% to 200%. The smallest increase was found in the only study showing an increase during recovery, while the greatest increase was found following an upper body only resistance exercise protocol(5). Only one investigation using the AHREP measured the change in monocytes, demonstrating an approximate 60% increase immediately after conclusion of the exercise bout(41).

Lymphocytes

Lymphocytes are a family of leukocytes consisting of T cells, B cells and Natural Killer (NK) cells. Together they represent 20-40% of circulating leukocytes, with T cells being the most prevalent(35). NK cells are a component innate immune system responsible for the lysis and apoptosis of infected cells as well as the production of cytokines IFN- γ and IL-10. B cells are component of the adaptive immune system and humoral immunity, whose functions include antigen presentation and the production of antibodies. T cells are also a component of the adaptive immune system and cell-mediated immunity with functions dependent on subtype. Cytotoxic T cells release apoptosis inducing enzymes, while T helper cells produce and secrete numerous cytokines(21, 35).

The lymphocyte response to exercise can be reported using total lymphocyte values or on a subset basis. Though the magnitude of response varies between lymphocyte subsets, the response pattern is similar across subsets and to that of total lymphocytes. In response to aerobic exercise, lymphocytes increase significantly during exercise with the magnitude of change greatest in NK cells and least in B cells. As with neutrophils, the rapid increase in lymphocytes is due to increased blood catecholamine values. Exercise duration, intensity and subject training status may affect the magnitude of lymphocyte response(21, 35).

Investigations of the lymphocyte response to resistance exercise show a similar response pattern to aerobic exercise, namely a significant increase in T cells, B cells, NK cells and total lymphocytes immediately after the RE bout(10,

41, 44, 54, 61). The magnitude of lymphocytosis following the resistance exercise bout differs between studies, likely due exercise intensity differences. Immediately after the bout, total lymphocytes increased approximately 30% - 100%, T cells increased 30%-60%, B cells increased 25%-80%, and NK increased 115%-225%. Malm et al suggested that B cells were more responsive to anaerobic and eccentric exercise, and the results of these resistance exercise investigations seem to support that hypothesis(35).

The limited recovery time points examined in the aforementioned investigations make analysis of lymphocyte values in recovery difficult. In these investigations, lymphocyte values appear to return to baseline 15-45 minutes after the resistance exercise bout. Evidence of lymphopenia is even more limited, though Nieman et al showed demonstrated significantly decreased total lymphocytes, T cells and NK cells 120 minutes after an acute resistance exercise bout(44). Data from Ramel et al seemingly indicate a return to baseline in total lymphocytes by 30 minutes post, with significantly decreased NK cells at 30, 60 and 120 minutes post, however, pairwise comparisons were not made(54).

Interestingly, the only investigation using solely upper body exercise failed to elicit a significant increase in circulating lymphocytes, despite increases in neutrophils and monocytes(5). The upper body protocol failed to induce any change in cortisol, and lactate values were not reported. Together, these findings support the importance of exercise intensity in eliciting a lymphocyte response.

Multiple investigations have used the AHREP to study the lymphocyte response. In Miles et al, 29 untrained women experienced increases in total

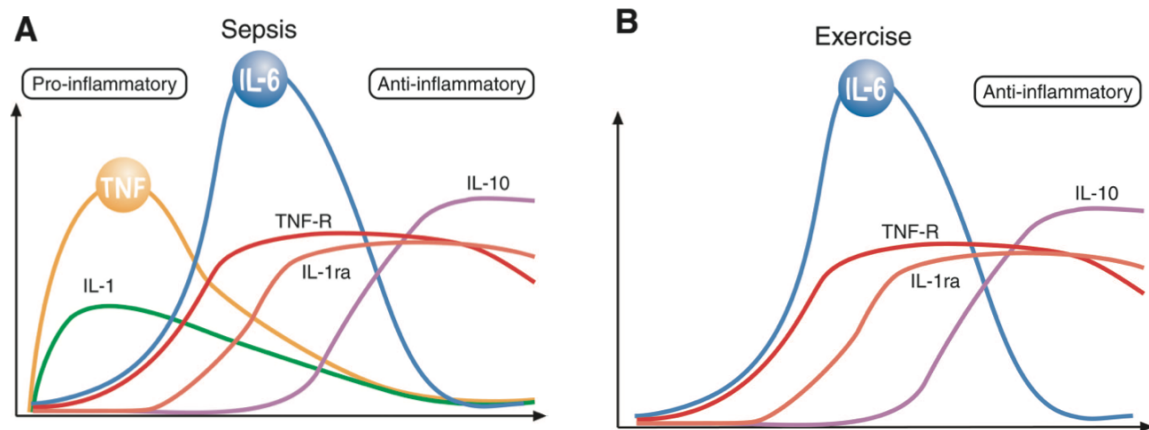
lymphocytes, T cells, B cells and NK cells of 52%, 36%, 31% and 184%, respectively(41). Dohi et al also used female subjects, and compared the lymphocyte response in the eight strongest women to that of the eight weakest women. The high strength group demonstrated increases in T cells, B Cells and NK cells of 53%, 57% and 152%, while the low strength group demonstrated increases 41%, 25% and 200%(10). These findings support the role of training status in the lymphocyte response. A second investigation by Miles et al analyzed the effect of training on the lymphocyte response to resistance exercise. Furthermore, subjects were divided into two groups based on the magnitude of post resistance exercise lactate values. Both groups demonstrated increases in all lymphocyte subsets following the AHREP, but training did not affect the response(40). Significant differences were observed between the high and low lactate groups for T cell and B cell responses(40).

The Effects of Stress on Cytokine Signaling

Cytokines are a diverse family of small glycoproteins that play a pivotal role in intercellular signaling, particularly in immune and inflammatory responses. In contrast with hormones, cytokines are effective at very small concentrations and often act in a paracrine or autocrine manner. These signaling molecules are produced and secreted by numerous sources including cells of the immune system, endothelial cells, smooth muscle cells, fibroblasts, skeletal muscle and adipose tissue(35, 64). Cytokines produced by skeletal muscle and adipose tissue are referred to as myokines and adipokines, respectively(55). Cytokines

can be pleiotropic, exerting different effects on different cells, and redundant, possessing overlapping functions. Additionally, interactions between cytokines can be synergistic or antagonistic(35). In response to various stresses and inflammatory stimuli, inflammatory cytokine production is upregulated, exerting significant influence on immune cell proliferation, differentiation, mobilization and functional capacity. The cytokine response differs greatly depending on the stimuli and can result in a systemic inflammatory response, as seen in sepsis, or more acute inflammation as seen following exercise(51). An example of the different responses to these stimuli is depicted in the figures below. This review will focus on the inflammatory cytokines IL-6, IL-10, IL-1 β , IL-12p70 and TNF- α .

Figure 2. Cytokine response pattern in sepsis and exercise



IL-6

Lymphocytes, monocytes, macrophages, fibroblasts and endothelial cells are the main sources of IL-6(35). Additionally, both adipose tissue and skeletal muscle produce and secrete IL-6(35). Skeletal muscle is thought to be the

predominant source of increased IL-6 following exercise (50). Production of IL-6 is stimulated by antigenic and mitogenic stimuli, lipopolysaccharide, viruses, and platelet derived growth factor(7, 35). Additionally, production can be up-regulated by other cytokines including IL-1, IL-2, and TNF- α . In certain situations, IL-1 and IL-13 can inhibit the production of IL-6(35).

IL-6 is considered both a pro-inflammatory and anti-inflammatory cytokine. The pro-inflammatory functions include stimulation of B cell differentiation and antibody production, T cell proliferation, differentiation and activation, neutrophil mobilization and functional augmentation and induction of acute phase protein synthesis by hepatocytes(7). In contrast, IL-6 exerts an anti-inflammatory influence by inhibiting the release of the pro-inflammatory cytokines, IL-1 β and TNF- α , as well as stimulating the release of soluble TNF receptor and the anti-inflammatory cytokines, IL-10 and IL-1ra(7, 35). IL-6 also exerts a significant impact on the endocrine system by stimulating production of ACTH in the anterior pituitary, inducing glucocorticoid production. The increase in glucocorticoids inhibits macrophage production of IL-6, providing a negative feedback mechanism(42).

In response to aerobic exercise, IL-6 increases more than any other cytokine(64), with increases as high as 128 fold observed following a marathon(48). Investigations have consistently observed IL-6 increases following aerobic exercise that increase exponentially with exercise duration(36, 51). The intensity of the exercise bout also has been shown to affect the magnitude of IL-6

increase(36, 51). Peak IL-6 is observed immediately after the exercise bout and decreases gradually in recovery(13).

Results from resistance exercise investigations are less conclusive, with both no change and significant increases observed (11, 24, 26-28, 52, 62, 68). Additionally, the time at which IL-6 increases are observed varies between investigations. When observed, the increases following resistance exercise are generally of a lower magnitude than that observed with aerobic exercise. Details of the resistance exercise investigations are found in Table 1.

IL-10

T cells, specifically T helper 2 cells, are the primary source of IL-10, though it is also produced by other T cell subtypes, as well as B cells, macrophages, dendritic cells, mast cells, NK cells, neutrophils and eosinophils(35, 42, 59). IL-10 functions as a strong anti-inflammatory agent, inhibiting the synthesis of numerous pro-inflammatory mediators such as TNF- α , IL-1, IL-8, IL-12, IFN- γ , G-CSF and GM-CSF and stimulating synthesis of IL-1ra and soluble TNF receptor(42, 59). Additionally, IL-10 can exert significant influence on macrophages, reducing MHC class II expression and production of nitric oxide and free radicals(35).

The IL-10 response to exercise is highly variable between individuals. As such, the effects of exercise on IL-10 are inconclusive. Marathon running has consistently resulted in increased IL-10, while shorter duration aerobic protocols have observed no change or smaller magnitude increases(35, 64).

Investigations employing resistance exercise have observed both IL-10 increases and no change, seemingly in equal proportion (11, 24, 26-28, 52, 62, 68, 70). As with IL-6, differences in exercise protocols and the recovery time points measured make comparisons difficult. Unlike IL-6, IL-10 increases have been observed in both trained and untrained individuals. Details of the resistance exercise investigations are found in the Table 1.

TNF- α , IL-1 β , IL-8, IL-12p70

With minor exceptions, resistance exercise has been shown to exert minimal if any influence on plasma concentrations of TNF α , IL-1 β , IL-8, IL-12p70, and therefore will be reviewed briefly.

TNF- α is a pro-inflammatory cytokine produced by monocytes, macrophages, neutrophils, activated lymphocytes, NK cells, endothelial cells and smooth muscle cells. TNF- α is also an adipokine, produced and secreted by adipose tissue. Secretion of TNF- α is induced by LPS, viruses, IL-1 and IL-2. In local inflammation, TNF- α stimulates neutrophil and macrophage infiltration. Numerous aerobic exercise investigations have found no change in TNF- α with exercise. Often, concentrations have been below detectable limits. When changes were observed, the increase was small and occurred immediately after exercise or several hours into recovery(33, 35, 42, 64).

IL-1 β is pro-inflammatory cytokine synthesized as a pro-peptide and is then proteolytically cleaved by IL-1 β converting enzyme (ICE) to produce the active form. Activated monocytes, macrophages synthesize the pro-protein in response to TNF- α . At relatively low concentrations, IL-1 β can stimulate the

release of TNF- α , IL-2, IL-4, and IL-6. As with TNF- α , high concentrations of IL-1 β can induce the production of acute phase proteins as part of a systemic inflammatory response. A few investigations have observed increased plasma IL-1 β concentrations immediately following long duration aerobic exercise, however the majority of investigations have not observed any change(35, 64).

The chemokine IL-8 recruits neutrophils to inflammatory sites and mediates their activation, resulting in its classification as a pro-inflammatory cytokine. Aside from its chemotactic role, IL-8 promotes angiogenesis. Endothelial cells, fibroblasts, monocytes, macrophages, neutrophils and T lymphocytes constitute the primary sources of IL-8. Marathons have been shown to increase IL-8 concentrations as much as 6 fold, whereas shorter, more moderate aerobic exercise results in smaller increases or no change(7, 35, 64).

IL-12p70 is a heterodimer cytokine produced by macrophages and dendritic cells in response to LPS and other antigen-presenting cell activating substances. IL-12p70 can affect inflammation by promoting the differentiation of T-cells to IFN- γ producing TH1 cells. Additionally, it affects NK cells by inducing proliferation and enhancing cytotoxic function. In aerobic exercise studies, IL-12p70 has been shown to decrease or show no change(1, 64, 66).

Table 1. The Effects of Resistance Exercise on Circulating Cytokines

Study	n	Subjects	Exercise	Cytokines Measured	Time points	Results
Fatouros et al (2010)	17	Untrained	Whole body exercise circuit (10 exercises, 1 set per circuit, 3 circuits)	IL-6, IL-10, IL-8, IL-1 β , TNF- α	PRE, 10M, 20M, 30M	\uparrow TNF- α (IP)
Hirose et al (2004)	10	Untrained	Elbow flexor eccentrics 40% 1RM 6 sets of 5 2 min rest	IL-6, IL-10, IL-8, IL-1 β , TNF- α	Pre, IP, 1H, 3H, 6H, 24H, 48H, 72H, 96H	\downarrow IL-8 (6H, 24H, 48H, 72H, 96H) \downarrow TNF- α (24H, 72H, 96H)
			Same exercise 4 weeks later	IL-6, IL-10, IL-8, IL-1 β , TNF- α	Pre, IP, 1H, 3H, 6H, 24H, 48H, 72H, 96H	\downarrow IL-8 (1H, 6H, 48H, 72H, 96H)
Izquierdo et al (2009)	12	Untrained	5x10 leg press at 10RM (Pre training)	IL-6, IL-10, IL-1 β	Pre, Mid, IP, 15m, 45m	\uparrow IL-6 (45m) \uparrow IL-1 β (mid, IP)
			5x10 leg press at 10RM (Post Training - Absolute load)	IL-6, IL-10, IL-1 β	Pre, Mid, IP, 15m, 45m	No observed changes
			5x10 leg press at 10RM (Post Training - Relative load)	IL-6, IL-10, IL-1 β	Pre, Mid, IP, 15m, 45m	\uparrow IL-6 (45m) \uparrow IL-10 (mid, IP, 15m, 45m)
Jajtner et al (2014)	10	Trained (control)	4 sets squat (80% 1RM), dl (70%), barbell split squat (70%) 90 sec rest, as many reps as possible, no more than 10	IL-6, IL-10	Pre, IP, 30m, 24H, 48H	\uparrow IL-10 (30m)
Kanda et al (2013)	9	Untrained	Calf raises 10 sets of 40 reps	IL-6, IL-10, IL-8, IL-1 β , IL-12p70 TNF- α	Pre, 2H, 4H, 24H, 48H, 72H, 96H	No observed changes
Smith et al (2000)	6	Untrained	Eccentric bench press and leg curl 4 sets of 12 reps at 100% full rep	IL-6, IL-10, IL-1 β , TNF- α	Pre, 1.5H, 6H, 12H, 24H, 48H, 72H, 96H, 120H, 144H	\uparrow IL-6 (12H, 48H, 72H) \uparrow IL-10 (72H, 96H, 144H) \downarrow IL-1 β (6H, 24H, 120H)
Tseng et al (2013)	11	Basketball Players	Elbow flexor extensors 6 sets of 5 at 85% 1rm 2 min rest	IL-6, IL-10, IL-8, IL-1 β , IL-12p70 TNF- α	Pre, IP, 3H, 24H, 48H, 72H	\uparrow IL-6 (IP, 3H, 24h, 48H, 72H)
Uchida et al (2009)	35	Soldiers	Bench press 4 exercise groups (50% 1rm 4x20) (75% 1RM 5x11) (90% 10 sets of 4) (110% 8x4 ecc only)	IL-6, IL-1 β , TNF- α	Pre, 24H, 48H, 72H	No observed changes
Phillips et al (2010)	14	Untrained	Eight exercise total body workout (LO group - 65% 1rm 2x12 and 3 set to failure) (HI group - 85% 1rm 2x8 3 set to failure)	IL-6	Pre, IP, 6H	\uparrow IL-6 (IP)

HSP70: A pleiotropic protein

Heat shock proteins (HSPs) are a group of highly conserved, ubiquitously expressed proteins classified into families according to molecular weight. These families include HSP90, HSP70, HSP60, HSP40 and small HSPs. Generally, HSPs act as cell chaperones, aiding in transport, protein folding and the prevention of aggregation of misfolded proteins. Location in the cell varies, and different families of HSPs possess divergent roles. HSP90 is a cytoplasmic and nuclear chaperone facilitating proper protein folding and protein degradation as well as maintaining the proper binding states of steroid hormone receptors. Located in the mitochondria, HSP60 assists in the assembly of mitochondrial proteins and transports proteins across mitochondrial membranes. HSP40 acts as a co-chaperone with HSP70, assisting in the formation of complexes and regulating HSP70's ATPase activity. The small HSPs, including $\alpha\beta$ -Crystallin and HSP27, help stabilize microfilament proteins, in addition to their chaperone functions, and therefore may play a role in the protection against, and adaptation to mechanical stress(43, 75).

HSP70 aids in the folding of newly synthesized proteins, helps refold misfolded proteins, prevents aggregation of misfolded proteins, interacts with cell signaling pathways, and plays a role in the regulation cell death(4, 23, 43, 75)The HSP70 family includes four different proteins, mitochondrial HSP70, Binding Immunoglobulin Protein, HSC70 and inducible HSP70. Of the four isoforms, HSC70 and inducible HSP70 are the most prominent. HSC70, also known as heat shock cognate protein or HSP73, is a constitutively expressed form of

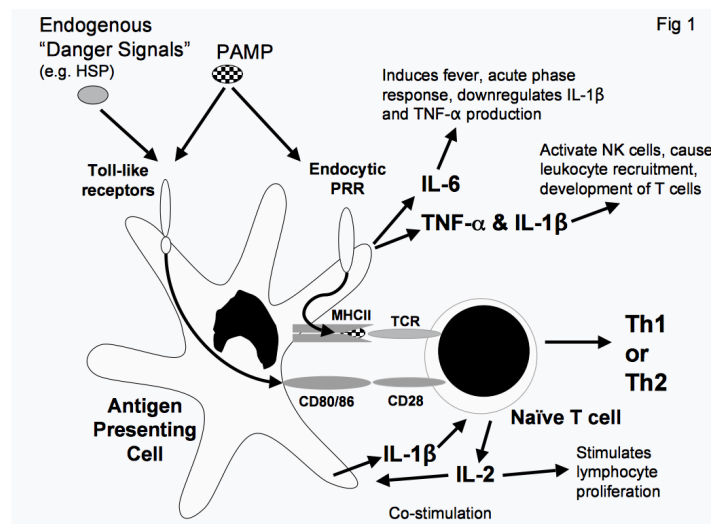
HSP70 with minimal stress response. In contrast, inducible HSP70 is expressed at low levels under non-stressed conditions, but exhibits a significant, stress inducible upregulation in response to heat, oxidative stress, ischemia, hypoxia, acidosis, increased intracellular Ca^{2+} and low glucose availability(43, 75). Given the association between exercise and several of these stressors, the response of inducible HSP70 to exercise has been the focus of numerous investigations.

Exercise stimulates two distinct inducible HSP70 responses, intracellular and extracellular, possessing paradoxical functions. Upregulation of HSP70 intracellularly is anti-inflammatory, anti-apoptotic, and associated with the more typical chaperone functions of HSPs(23). The increased expression of intracellular HSP70 (IC HSP70) exerts a cyto-protective effect on the cell, attenuating the damage of future insults. Numerous studies have shown that IC HSP70 increases dramatically in response to aerobic and resistance exercise in leukocytes and skeletal muscle. Furthermore, training studies have generally shown that in trained individuals resting concentrations of IC HSP70 are lower, but HSP70 mRNA is increased, allowing for a greater and faster HSP70 response to stress.

Less studied is the increase in extracellular HSP70 (eHSP70) in response to exercise and the corresponding effect on immune cells. In vitro investigation, Asea et al demonstrated that eHSP70 could stimulate the production of the pro-inflammatory cytokines IL-1 β , TNF- α , and IL-6(2). Consequently, high concentrations of eHSP70 in serum have been associated with various pathologies such as hypertension and vascular disease(45, 47). A conflicting

observation was made by Detanico et al, who found that eHSP70 could stimulate production of the anti-inflammatory cytokine, IL-10(9). Some researchers attribute the conflicting findings to the recombinant HSP70 used by Asea et al, which may have been contaminated with LPS(67). The cytokine stimulating mechanism of eHSP70 is depicted below.

Figure 3. HSP70 as a danger signal to stimulate cytokine production



eHSP70 has also been shown to stimulate proliferation of natural killer cells and neutrophil chemotaxis and phagocytosis(4). Additionally, eHSP70 affects the adaptive immune system through stimulation of dendritic cell and macrophages and their presentation of antigens(71). Though the exact mechanisms causing eHSP70 concentrations to increase remain to be elucidated, in vivo release of HSP70 from the brain and liver have been demonstrated(12, 34). Furthermore, in vitro studies have identified glial cells,

peripheral blood mononuclear cells, and neutrophils as potential sources of eHSP70(20, 25).

A significant increase in eHSP70 has been thoroughly demonstrated following acute aerobic exercise bouts(43, 75), however only three studies to date have investigated the eHSP70 response to resistance exercise modalities. A training study using 85-year old women showed that 12 weeks of low intensity resistance training led to decreases in resting eHSP70 in correlation with decreases in inflammatory markers(45). The two investigations analyzing the affects of an acute bout of resistance exercise failed to identify any significant increase in eHSP70(24, 46). The resistance exercise stimulus, in both investigations, was low to moderate intensity elbow flexor eccentrics performed by untrained individuals. Both exercise duration and intensity are thought to impact the eHSP70 response, and norepinephrine has been proposed as a potential stimulus for eHSP70 secretion(20), therefore, a more taxing resistance exercise protocol may demonstrate an eHSP70 increase.

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Chapter 3: Methods

Subjects

Eleven resistance trained men (23.6 ± 4.5 yr, 176.3 ± 5.5 cm, 86.0 ± 12.7 kg) participated in the study. All subjects had minimum of one year of resistance training experience and were able to squat at least 150% of their body weight on a smith machine. Prior to participation, all subjects were informed of the procedures, benefits and potential risks and signed a consent form in accordance with the guidelines of the University of Connecticut Institutional Review Board for the use of human subjects. All subjects completed a medical history questionnaire and were cleared of any injuries or medical complications that could confound the results of the investigation. The University of Connecticut Institutional Review Board approved this study.

Study Design

The study consisted of five visits including a familiarization, an acute heavy resistance exercise protocol (AHREP) and three recovery visits: 24, 48 and 72 hours after the AHREP.

Familiarization Visit

During the familiarization visit, subject anthropometric data was recorded, subjects were familiarized with the various tests and performance measures, and a smith machine back squat one repetition maximum (1RM) was determined. Additionally, a full AHREP was performed.

AHREP and Recovery Visits

Upon arrival for the AHREP and recovery visits, subject hydration status was assessed via urine specific gravity (USG) using a refractometer (Reichert, Lincolnshire, IL). Subjects with a USG of ≤ 1.025 were considered adequately hydrated. At the beginning of the AHREP visit subjects performed five minutes of low-intensity cycle ergometry followed by an established dynamic warm up protocol. Blood was sampled at various time points throughout the AHREP visit, as well as at each of the recovery visits.

Subjects performed six sets of 10 repetitions of back squats to parallel on a smith machine, allowing only vertical translation of the bar. The starting weight was approximately 75% of the 1RM, as determined on the familiarization day. If a subject was unable to complete all 10 repetitions, the weight was reduced at the discretion of the testers, and the remaining repetitions were completed. The weight was only reduced to allow for completion of the required repetitions, with the goal of completing the AHREP with highest possible load volume. Rest periods of two minutes were given following sets one and two, while rest periods of three minutes were given following sets three, four and five. Pilot testing indicated that the additional minute of rest allowed subjects to complete the AHREP with a higher load.

Blood draws

On the day of the AHREP an indwelling cannula was inserted into a

superficial forearm vein. Blood samples were obtained immediately before (PRE) and after the protocol (IP), and 15 (15m), 30 (30m), 60 (60m) and 120 (120m) minutes into recovery. A blood sample was collected at the 24 (24h), 48 (48h) and 72 (72h) hour recovery visits.

Biochemical analysis

Whole blood was analyzed for complete blood count with differential (includes red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, white blood cell count, absolute count and percent neutrophil, lymphocyte, monocytes, eosinophils, and basophils, platelet count, and mean platelet volume) via an automated hematology analyzer with by Quest Diagnostics (Madison, NJ). A cytometric bead array (Becton Dickinson, San Jose, CA) was used to analyze plasma IL-1 β , IL-6, IL-8, IL-10, IL-12p70 and TNF- α , in accordance with the manufacturer's instructions. The standards were fitted to a quadratic equation via FCAP software and mean fluorescent intensity for the samples was interpolated.

The creatine kinase-SL assay (SEKISUI, Charlottetown, Canada) was performed in duplicate on human serum samples. The assay wavelength was read at 340 nm (Biomate3 Spectrophotometer; Thermo Scientific, Pittsburgh, PA). The coefficient of variation (CV) was 4.2%. Lactate was measured in duplicate from EDTA-plasma samples using a liquid lactate reagent (Point Scientific, Canton, MI) and assayed according to Gutmann et al. and Noll et al.

with modifications. Intra-and inter-assay coefficients of variations (CV) for lactate were below 3.9%. Cortisol was measured in duplicate from serum using an ELISA (CALBiotech, Spring Valley, CA), with a sensitivity of 11.1 nmol/L. Intra-and inter assay CVs for cortisol were below 7.2 All ELISAs and lactate assays were measured on a Versamax tunable microplate reader (Molecular Devices, Sunnyvale, CA) at the appropriate wavelength for that particular assay.

Statistical Analysis

Data was analyzed over time using a repeated measures ANOVA. When an effect for time was detected, pairwise comparisons were made using Fisher's LSD using statistical software (IBM SPSS Statistics Version 21, Armonk, NY). Significance was set at $p \leq 0.05$. All data is presented as mean \pm standard deviation.

Chapter 4: Results

The AHREP produced significant amounts of systemic stress, as indicated by acute post exercise increases in lactate, cortisol, and prolonged increases in CK. Following the AHREP, lactate was elevated at all acute time points. Lactate concentrations were greatest at immediately after exercise (IP), and decreased gradually throughout recovery. Cortisol was elevated at IP, and continued to increase to peak values at 30m, before returning to PRE values by 60m. When compared to pre-AHREP values, no differences in cortisol values were observed at 24H, 48H or 72H. CK elevations were highest twenty-four hours after exercise (24H), remained elevated at 48H, and returned to PRE values by 72H. Means and SD for the aforementioned biomarkers are reported in Table 2.

Absolute leukocyte counts changed following the AHREP. Neutrophilia was observed at all acute timepoints. Neutrophils peaked at IP, decreased at 15 and 30m, and increased again at 60m and 120m. Lymphocytosis was observed at IP and 15m. Following the peak at IP, lymphocytes decreased rapidly, resulting in lymphocytopenia at 30m, 60m and 120m. Monocytosis was also observed at IP and 15m, with the peak elevation occurring at IP. Monocytes returned to PRE values by 30m, decreased below PRE values at 60m and returned to PRE values at 120m. Eosinophils were elevated at IP and decreased throughout recovery, resulting in eosinopenia at 60m and 120m (compared to PRE values). Basophils were elevated at 15m, but similar to PRE values at all other time points. At 24H, no differences were observed in any of the leukocytes.

However, at 48H there was a trend for an increase in monocytes, and monocyte counts were elevated above PRE values at 72H. Absolute leukocyte values are reported in Table 3.

We observed significant increases in eHSP70 immediately after the AHREP, which remained elevated at 15m before returning to PRE values at 30m. No differences were observed at 24H, 48H or 72H. eHSP70 values are displayed in Figure 4.

No differences were observed in IL-1 β , IL-6, IL-8, IL-10, IL-12p70 or TNF- α at any time point. Results are displayed in Figure 5.

Table 2. Circulating Measures of Stress and Tissue Damage Following Acute Heavy Resistance Exercise Protocol

Measure	PRE	IP	15m	30m	60m	120m	24H	48H	72H
Lactate	0.9 ± 0.3	12.1 ± 3.2*	9.1 ± 2.8*	6.1 ± 1.9*	2.9 ± 0.8*	1.4 ± 0.4*			
Cortisol	494 ± 114	671 ± 159*	717 ± 189*	667 ± 198*	450 ± 123	321 ± 83*	502 ± 195	546 ± 146	544 ± 138
Creatine Kinase	126 ± 29						449 ± 147*	299 ± 130*	151 ± 50

Values are reported as means ± SD. * Significantly different from PRE at $P \leq 0.05$.

Table 3. Circulating Leukocyte Counts Following Acute Heavy Resistance Exercise Protocol

Circulating leukocyte counts in response to a heavy acute resistance exercise protocol.

Measure	PRE	IP	15m	30m	60m	120m	24H	48H	72H
Neutrophils	3353 ± 1674	5548 ± 2739*	4479 ± 1991*	3898 ± 1693*	4138 ± 1990*	5512 ± 2877*	3978 ± 2158	3497 ± 1592	3336 ± 1792
Lymphocytes	1996 ± 624	4108 ± 1337*	2635 ± 1040*	1540 ± 483*	1194 ± 303*	1261 ± 312*	1645 ± 550	1813 ± 529	1687 ± 363
Monocytes	478 ± 126	1001 ± 317*	647 ± 225*	451 ± 112	414 ± 106*	494 ± 132	485 ± 144	545 ± 168	551 ± 132*
Eosinophils	206 ± 93	327 ± 163*	242 ± 131*	192 ± 92	141 ± 68*	111 ± 43*	200 ± 83	188 ± 82	194 ± 80
Basophils	27 ± 14	37 ± 13	38 ± 13*	30 ± 14	23 ± 9	21 ± 13	29 ± 9	24 ± 11	23 ± 10

Values are reported as means ± SD. * Significantly different from PRE at $P \leq 0.05$.

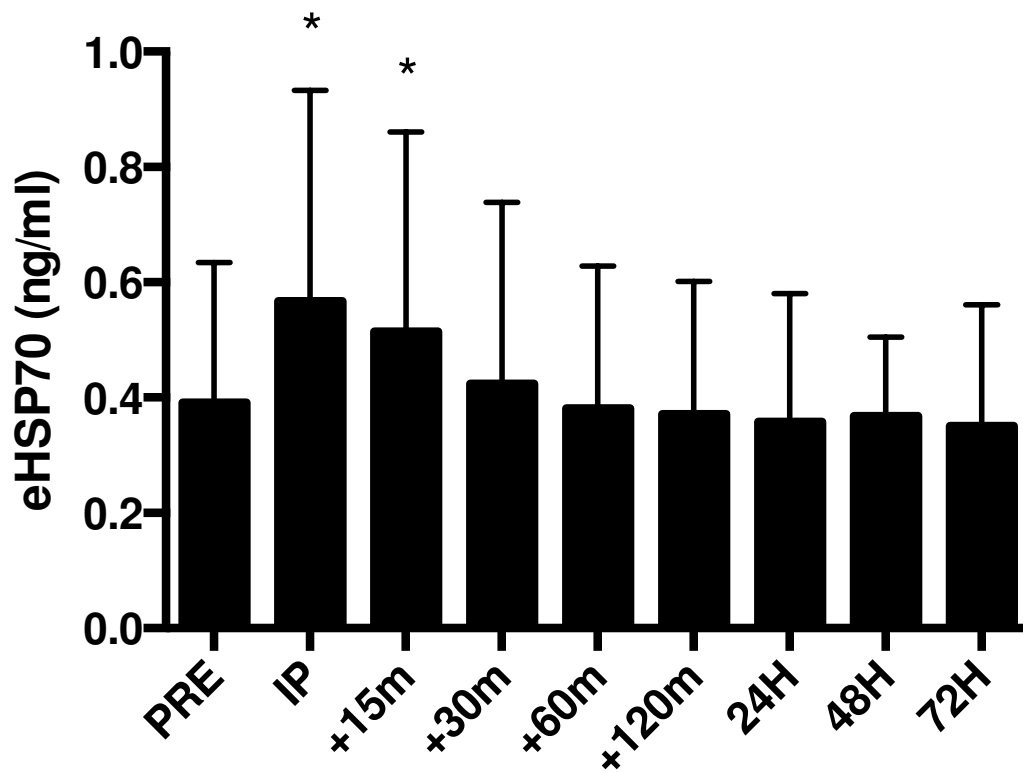


Figure 4. eHSP70 Response to Acute Heavy Resistance Exercise

Data are presented as means \pm SD. We observed significant increases from PRE at the immediate post (IP) and 15 minute post (+15m) time points. * Significantly greater than PRE at $p \leq 0.05$.

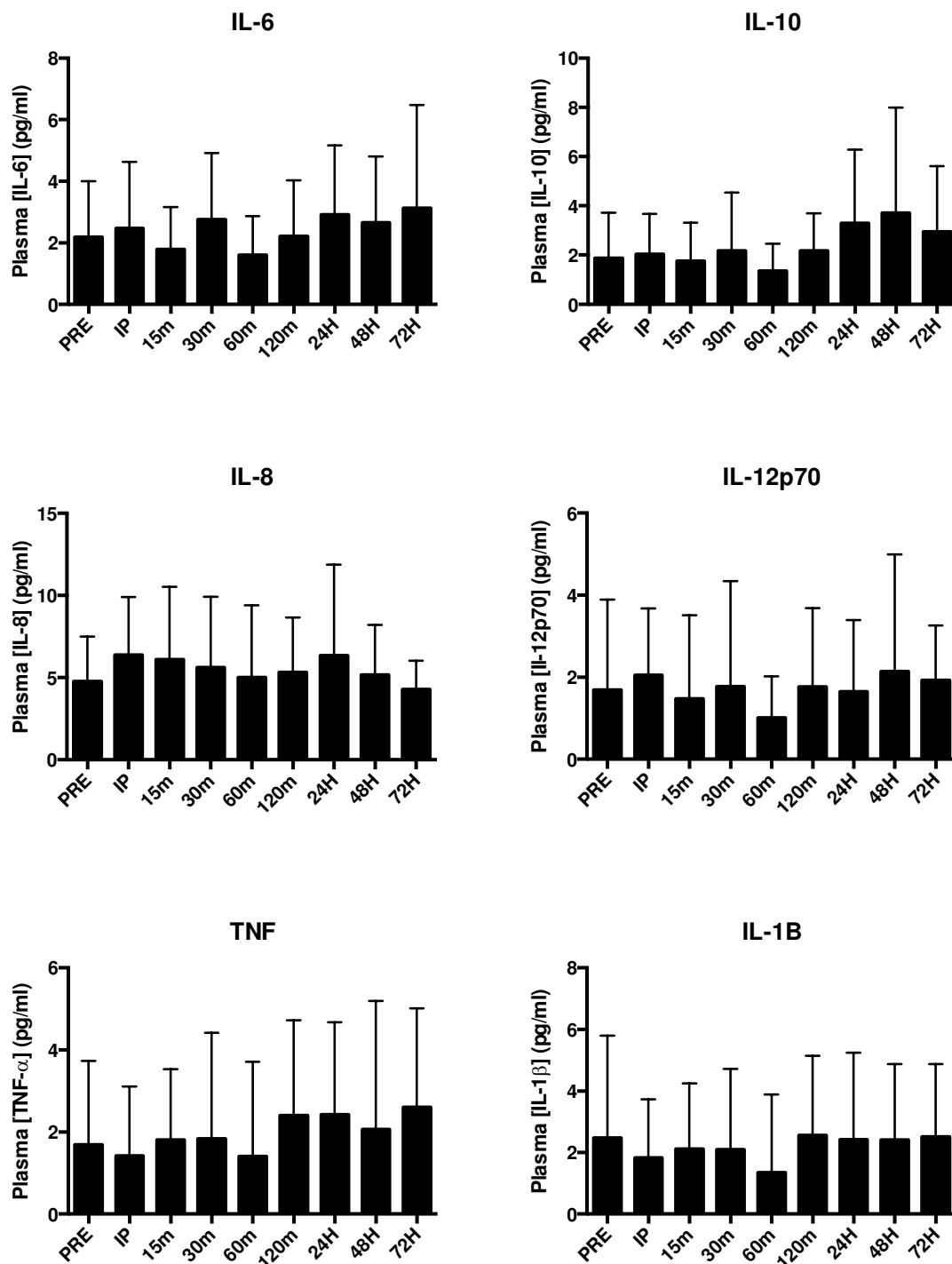


Figure 5. Circulating Cytokines Unchanged after Heavy Resistance Exercise

Data are presented as means \pm SD. We did not observe significant increases or decreases from PRE for any of the measured cytokines.

Chapter 5: Discussion

Previous studies investigating the effects of acute resistance exercise on inflammatory and immune responses have employed a wide variety of exercise protocols utilizing different movements, intensities, volumes and rest periods. Consequently, the immune and inflammatory responses, as measured by circulating leukocytes and inflammatory cytokines, have also varied widely. Differences in subject training status may also contribute to these findings. We employed the AHREP, a high intensity, high volume squat protocol that causes significant metabolic, adrenergic, hormonal and mechanical stress as exemplified by significant changes in lactate, catecholamines, cortisol, myoglobin and creatine kinase in resistance-trained men. Taken together, we can conclude that the AHREP exerts a substantial, multifaceted stress upon even resistance-trained individuals.

Aerobic training has been shown to elicit a biphasic neutrophilic response, with peaks immediately after the protocol and again in recovery(14, 18, 30). While resistance training has been consistently shown to induce neutrophilia, the response pattern is less clear due to the limited recovery time points used in most studies. Though subjects in this investigation experienced sustained neutrophilia throughout all recovery time points, there were significant decreases in neutrophil counts between IP and 15m and between 15m and 30m. A second significant increase in neutrophils was observed between 60m and 120m, in accordance with the biphasic response observed in the aerobic training investigations. Others have suggested that acute and delayed neutrophil peaks

are the result of increased catecholamine and cortisol concentrations, respectively(30). An investigation by Ramel et al observed a similar response, though neutrophil counts at the 120m recovery time point were greater than what was observed immediately after the exercise protocol(40). Had the recovery time point extended beyond 120m, it is possible that the delayed neutrophil increase may have exceeded that of the initial peak. The responses of lymphocytes, monocytes, eosinophils and basophils are consistent with previous investigations(6, 12, 29, 32, 35, 40, 42).

eHSP70 has consistently been shown to increase in response to aerobic exercise(8, 27, 49). However, previous investigations failed to show an increase in eHSP70 following resistance exercise(20, 37). In this investigation, a stressful resistance exercise protocol elicited an increase in eHSP70 in the absence of other direct environmental stressors. Immediately following the AHREP, eHSP70 values were elevated above pre-exercise values and remained elevated 15 minutes into recovery before returning to baseline levels by the 30m time point. The increase in eHSP70 as a result of exercise is intensity dependent, as demonstrated by Fehrenbach et al, who compared treadmill runs of equal duration at 80% and 60% of VO₂max, and observed a significantly greater eHSP70 increase at the higher intensity(9). Differences in exercise intensity between this investigation and previous resistance exercise investigations may explain the conflicting results. Unfortunately, neither of the previous investigations reported lactate values. The inability of the second exercise bout to elicit an increase in CK in untrained subjects in the Hirose et al investigation, and

the minor norepinephrine increase observed in the Ogawa et al investigation are indicative of the relatively low intensities of the resistance exercise protocols(20, 37).

Exercise trained individuals have been shown to express lower concentrations of intracellular HSP70, but an increased intracellular HSP70 response to stress(17). Furthermore, resting concentrations of eHSP70 have been shown to decrease with increased physical activity(36). Given that our subjects were resistance-trained and exhibited relatively lower resting eHSP70 concentrations, we speculate that they may have experience a pronounced eHSP70 response when compared with untrained subjects used in previous investigations. Therefore, training status may provide an additional explanation for the observed differences.

The exact cause of exercise-induced increases in eHSP70 remains to be determined. *In vitro* studies using physiological concentrations have demonstrated the ability of norepinephrine to induce eHSP70 secretion from neutrophils(15, 19). While norepinephrine concentrations were not measured in the current study, investigations with similar protocols and subjects have demonstrated increases(11). As such, exercise induced increases in norepinephrine provide a possible explanation for increased eHSP70.

eHSP70 may exhibit cytokine-like function, as demonstrated *in vitro*. Asea et al confirmed the ability of eHSP70 to bind to monocytes and induce the production and secretion of pro-inflammatory cytokines, TNF- α , IL-1 β and IL-6(2). More recently however, eHSP70 has been shown to induce the secretion of

IL-10 from peripheral blood mononuclear cells, triggering anti-inflammatory processes(5). The lack of cytokine increases may be due to changes in TLR2 expression as a result of exercise. Stewart et al demonstrated that just 12 weeks of combined aerobic and resistance training was able to decrease the TLR2 expression of monocytes at rest by 31%(45). Additionally, TLR2 expression has been shown to decrease acutely following exercise (16, 26).

Though aerobic exercise routinely elicits an IL-6 increase (46) in an intensity and duration dependent manner(10), the effects of resistance exercise are inconclusive. Previous investigations have observed acute increases(22, 39, 43, 47) and no change (7, 20, 23, 24, 48). Additionally, initial increases in IL-6 were observed at different times: immediately post-exercise, 45 minutes into recovery, and 12 hours into recovery. The observed differences in IL-6 response may be related to muscle glycogen. Based on evidence that IL-6 secretion from muscle increases in glycogen depleted muscle(44) and decreases following glucose ingestion, Pedersen et al proposed that IL-6 acts as an “energy sensor” in the muscle(38). Since training results in increased muscle glycogen stores, subject training status may again explain observed differences in IL-6 responses to resistance exercise. This theory is supported by the findings of Jajtner et al, who failed to observe increases in IL-6 in resistance trained subjects, in addition to previous work using untrained subjects, where IL-6 increases were observed(23).

IL-10 was not elevated following the AHREP, likely due to an absence of IL-6 signaling. Previous resistance exercise investigations reporting increases in IL-10 have observed increases in IL-6, with the exception of Jajtner et al(23). In this investigation, significant variance in cytokine expression was observed between subjects. It is possible that Jajtner et al experienced similar variability resulting in no significant IL-6 increase despite elevated concentrations in some subjects. The rapid decrease of monocytes immediately after IP may additionally explain the lack of an IL-10 response, as monocytes are the primary producers of IL-10 in the acute inflammatory response.

In summary, we observed significant systemic stress in response to intense whole body resistance exercise. Leukocyte counts changed in accordance with previous literature, likely due to the affects of catecholamines and cortisol. To our knowledge, we are the first to report increased eHSP70 in response to resistance exercise. Increases in this “chaperokine” were not accompanied by changes in pro or anti-inflammatory cytokines. While unresolved, we suggest that this may be explained by training-induced increases in muscular glycogen content or by reductions in TLR2 expression. Finally, future efforts aimed at better understanding the role of eHSP70 should consider the apparent importance of using sufficiently stressful high intensity exercise.

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